15-Lipoxygenase-2 Expression in Benign and Neoplastic Sebaceous Glands and Other Cutaneous Adnexa

Scott B. Shappell, Diane S. Keeney,* Jing Zhang, Robert Page, Sandy J. Olson, and Alan R. Brash†
Departments of Pathology, *Biochemistry, and †Pharmacology, Vanderbilt University Medical Center, Nashville, Tennessee, U.S.A.

15-Lipoxygenase-2 has a limited tissue distribution in epithelial tissues, with mRNA detected in skin, cornea, lung, and prostate. It was originally cloned from human hair rootlets. In this study the distribution of 15-lipoxygenase-2 was characterized in human skin using immunohistochemistry and *in situ* hybridization. Strong uniform 15-lipoxygenase-2 *in situ* hybridization (n = 6) and immunostaining (n = 16) were observed in benign cutaneous sebaceous glands, with expression in differentiated secretory cells. Strong 15-lipoxygenase-2 immunostaining was also observed in secretory cells of apocrine and eccrine glands. Variable reduced immunostaining was observed in skin-derived sebaceous neoplasms (n = 8). In the eyelid, Meibomian glands were uniformly negative for 15-lipoxygenase-2 in all cases examined (n = 9), and sebaceous carcinomas apparently derived from Meibomian glands were also negative (n = 12). The mechanisms responsible for differential expression in cutaneous sebaceous *vs* eyelid Meibomian glands remain to be established. In epidermis, positive immunostaining was observed in the basal cell layer in normal skin, whereas five examined basal cell carcinomas were negative. Thus, the strongest 15-lipoxygenase-2 expression is in the androgen regulated secretory cells of sebaceous, apocrine, and eccrine glands. This compares with the prostate, in which 15-lipoxygenase-2 is expressed in differentiated prostate secretory cells (and reduced in the majority of prostate adenocarcinomas). The product of 15-lipoxygenase-2, 15-hydroxyeicosatetraenoic acid, may be a ligand for the nuclear receptor peroxisome proliferator activated receptor-γ, which is expressed in sebocytes, and contribute to secretory differentiation in androgen regulated tissues such as prostate and sebaceous glands. ***Key words: apocrine glands/arachidonic acid/Meibomian glands/peroxisome proliferator activated receptors. J Invest Dermatol 117:36–43, 2001***

The recently identified human lipoxygenase, 15-lipoxygenase-2 (15-LOX-2), has about 35–40% amino acid sequence identity to the previously characterized 5-LOX, 12-LOX, and 15-LOX (15-LOX-1) (Brash *et al*, 1997) and about 50% identity to the human 12R-LOX (Boeglin *et al*, 1998; Sun *et al*, 1998). 15-LOX-2 has a limited distribution in epithelial tissues (Brash *et al*, 1999b): the cDNA was originally cloned from human hair rootlets, and 15-LOX-2 mRNA was detected in skin, cornea, lung, and prostate (Brash *et al*, 1997; Kilty *et al*, 1999). In contrast to the previously known human lipoxygenases, 15-LOX-2 was not identified in peripheral blood leukocytes (Brash *et al*, 1997). We have demonstrated that 15-LOX-2 is uniformly expressed in the differentiated secretory epithelial cells of benign prostate and that benign prostate synthesizes the 15-LOX-2 metabolite 15S-hydroxyeicosatetraeinoic acid (15S-HETE) from arachidonic acid (Shappell *et al*, 1999). Furthermore, 15-LOX-2 is substantially or completely reduced in the majority of prostate adenocarcinomas (Shappell *et al*, 1999; Jack *et al*, 2000).

Manuscript received October 18, 2000; revised January 23, 2001; accepted for publication February 7, 2001.

Reprint requests to: Dr. Scott B. Shappell, C-3321, Medical Center North, Department of Pathology, Vanderbilt University Medical Center, Nashville, TN 37232-2561.

Abbreviations: 15-LOX-2, 15-lipoxygenase-2; PPARγ, peroxisome proliferator activated receptor-gamma.

15-Lipoxygenase is consistently reported as one of the main lipoxygenase activities in normal human skin (Nugteren and Kivits, 1987; Duell *et al*, 1988; Baer *et al*, 1991) and the major activity in human keratinocytes (Burrall *et al*, 1985, 1988; Green, 1989). 15-LOX is also a prominent activity in plucked human hair rootlets (von Henneicke-von Zepelin *et al*, 1991; Baer and Green, 1993). Based on cDNA cloning, the expression of both 15-LOX-1 and 15-LOX-2 is established in skin (Zhao *et al*, 1995; Brash *et al*, 1997). These two enzymes are likely to be functionally quite distinct, with different primary structures, substrate specificities and tissue distributions. 15-LOX-1, discovered originally in reticulocytes, is potentially involved in facilitating cell maturation (Schewe and Kühn, 1991; van Leyen *et al*, 1998; Brash, 1999), whereas 15-LOX-2 appears designed to synthesize 15-HETE exclusively, possibly as a specific ligand and signaling molecule (Brash, 1999).

Based on amino acid sequence identities of 78%, it appears that the structural homolog of 15-LOX-2 in the mouse is an 8-LOX (Jisaka *et al*, 1997; Krieg *et al*, 1998). These two enzymes share some unique structural aspects, such as a serine residue instead of a conserved histidine or asparagine in a position close to the iron in the catalytic domain (Jisaka *et al*, 1997). The mouse 8-LOX is known to be inducible in skin by topical application of phorbol ester (Gschwendt *et al*, 1986; Fürstenberger *et al*, 1991; Hughes and Brash, 1991) and is expressed in differentiated keratinocytes (Jisaka *et al*, 1997). The product of the mouse 8-LOX, 8-HETE, has been shown recently to activate peroxisome proliferator-activated

receptor (PPAR) -α and thereby induce differentiation in murine keratinocytes (Muga *et al*, 2000).

In vitro studies utilizing expression vectors indicate that 15-HETE may be able to activate transcription as a ligand for PPARγ (Nagy *et al*, 1998). We have recently demonstrated that 15-HETE activates PPARγ-dependent transcription and inhibits proliferation of prostate carcinoma cell lines (Shappell *et al*, 2001). Hence, 15-HETE may represent an endogenous ligand for PPARγ in tissues expressing 15-LOX-2. As 15-LOX-2 has a limited tissue distribution and was cloned from hair rootlets, in this study we have investigated its distribution in human skin. Our analysis has included sebaceous glands, as similar to the prostate, differentiation of sebocytes is regulated by androgens. Both prostate and sebocytes express androgen receptor and 5α-reductase, which converts circulating testosterone to dihydrotestosterone (Thody and Shuster, 1989; Luu-The *et al*, 1994; Russell and Wilson, 1994; Deplewski *et al*, 1997; Pelletier *et al*, 1998). Furthermore, PPARγ is expressed in sebaceous glands, and synthetic PPARγ agonists can induce sebocyte differentiation (Rosenfield *et al*, 1998). As 15-LOX-2 expression was reduced in prostate adenocarcinoma compared with differentiated benign prostate epithelium (Shappell *et al*, 1999; Jack *et al*, 2000), we have also begun exploring possible altered 15-LOX-2 expression in neoplastic *vs* benign sebocytes.

MATERIALS AND METHODS

Case selection Thirty-nine cases from 37 patients were retrieved from the files of the Surgical Pathology and Dermatopathology laboratories of Vanderbilt University Medical Center. Hematoxylin and eosin slides were reviewed and cases were selected for the presence of benign sebaceous, eccrine, and apocrine glands and to achieve a random mixture of male and female patients of various ages, skin sites, and different primary disorders for which the original surgical procedure or biopsy was performed. In addition to skin sebaceous glands, eyelid cases were selected to provide a source of benign Meibomian glands. There were 14 males and 23 females, with a mean age of 55.6 y (range 2–92) and a median age of 60 y. Specific skin or other sites (known for 35 of 37) were periorbital/eyelid 21, face (including ears, nose, lips) 11, chest/back two, and scalp one. Normal adnexa were from cases diagnosed as "sebaceous hyperplasia" (n = 3), and from miscellaneous other conditions, including squamous cell carcinoma or scar from squamous cell carcinoma or melanoma re-excision (n = 6), basal cell carcinoma (n = 5), intradermal nevus (n = 1), nevus sebaceous of Jadassohn (n = 1), ptosis repair (n = 2), and chronic conjunctivitis (n = 1). Benign and malignant neoplastic sebaceous lesions were selected from a search of the pathology files. Hematoxylin and eosin slides were reviewed and only cases that met well established criteria for the diagnoses of sebaceous adenoma (n = 4, from face and back) and sebaceous carcinoma (n = 16) were included. Sebaceous carcinomas represented lesions arising both from Meibomian glands (n = 12) and skin sebaceous glands (n = 4).

Immunohistochemistry Paraffin immunoperoxidase studies were performed on an automated immunostainer (Ventana ES automated immunohistochemistry system, Ventana Medical Systems, Tucson, AZ) with an avidin–biotin method without antigen retrieval techniques. The rabbit polyclonal anti-human 15-LOX-2 antibody employed was prepared against purified 15-LOX-2 protein as described (Shappell *et al*, 1999). Previous western blot studies have shown that this antibody reacts strongly to 15-LOX-2, without cross-reactivity to 15-LOX-1, 5-LOX, or platelet type 12-LOX, and with weak cross-reactivity to human 12R-LOX (Shappell *et al*, 1999). Immunostaining on paraffin sections is eliminated by prior antibody absorption with purified 15-LOX-2 protein (Shappell *et al*, 1999). Primary antibody was used at a 1 : 1000 dilution. Immunostaining in different skin compartments and adnexa is reported descriptively or semiquantitatively from 0 to 4+: 0, negative; 1+, focal faint, barely discernible; 2+, moderate intensity in approximately half of cells/glands; 3+, moderate staining in most cells/glands or strong immunostaining in approximately 75% of cells/glands; 4+, strong uniform immunostaining in essentially all cells/glands.

***In situ* hybridization** Discarded human skin tissues were obtained according to protocols approved by Vanderbilt University Institutional Review Board. Tissues were immersion-fixed overnight in 4% buffered paraformaldehyde. Processing for paraffin embedding and *in situ* hybridization were performed as described (Keeney, 1999). [^{35}S]-uridine triphosphate cRNA were produced by *in vitro* transcription from a 564 base pair 15-LOX-2 cDNA fragment, prepared from a full length 15-LOX-2 cDNA in pBluescript SK vector (Stratagene, La Jolla, CA) cut with *Eco*rV at position 1380 and *Xho*I at position 1944, generating a sequence unique to 15-LOX-2 (*vs* other lipoxygenases) (Brash *et al*, 1997). Specific hybridization was distinguished by comparing silver grain development produced by sense and anti-sense cRNA applied to consecutive sections on the same slides. The data presented are representative results obtained by screening several neonatal foreskins and breast and abdominal skin samples.

RESULTS

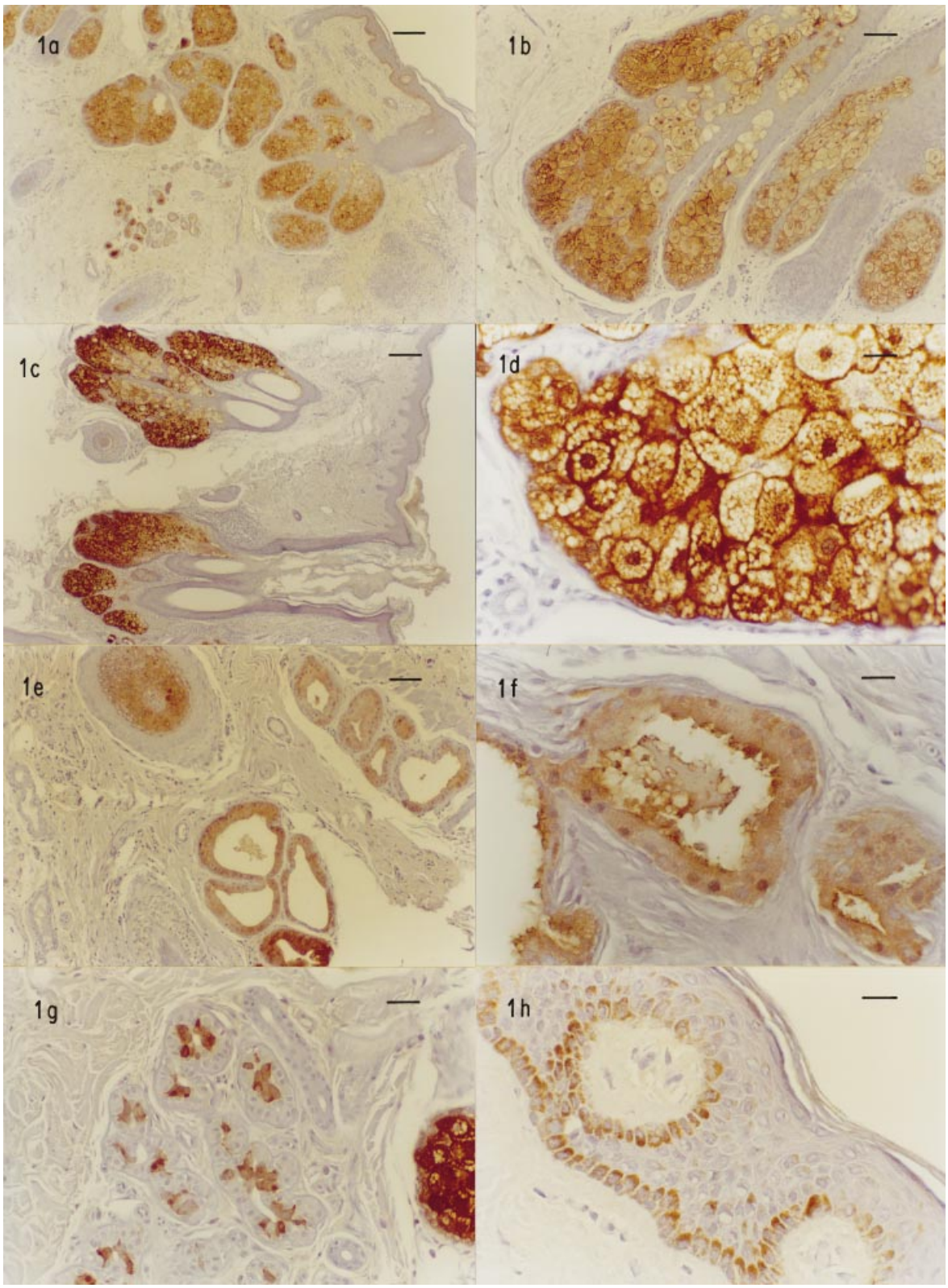
15-LOX-2 immunostaining in benign cutaneous sebaceous glands, other cutaneous adnexa, and epidermis Strong, uniform (4+) 15-LOX-2 immunostaining was observed in essentially all skin sebaceous glands in all cases (n = 16) (**Fig 1*a*–*d***). 15-LOX-2 immunostaining in sebaceous glands was noted in normal sebaceous glands in cases with unrelated lesions, in sebaceous glands in lesions diagnosed as sebaceous hyperplasia, and in a nevus sebaceous of Jadassohn. Strong cytoplasmic immunostaining was noted in differentiated sebaceous cells with abundant cytoplasm. Some nuclear staining was also noted (**Fig 1*d***). Immunostaining was generally not detected in undifferentiated basal cells of sebaceous glands (**Fig 1*b***), with weak to intermediate staining occasionally noted in "intermediate" cells towards the gland lumens, with more cytoplasm, but without prominent lipid accumulation (cytoplasmic vacuolation) (not shown). In contrast to the uniform expression of 15-LOX-2 in differentiated sebocytes, clear cells of the outer hair root sheath were generally negative (**Fig 1*b*, *c*, *e***), but staining was noted in the inner root sheath/hair matrix in two cases (**Fig 1*e***). The follicular epidermis was generally negative (**Fig 1*c***).

Positive 15-LOX-2 immunostaining was noted in other cells with secretory differentiation in other skin adnexa. Generally strong 15-LOX-2 immunostaining was present in apocrine glands, including those in periorbital tissues (present in nine of nine cases, 3 or 4+ in 7 and 2+ in 2) (**Fig 1*e*, *f***). Immunostaining was noted in the cytoplasm, with staining of lumen secretions and some nuclei as well. Strong 15-LOX-2 immunostaining was frequently observed in secretory (luminal) cells of eccrine glands (n = 20, with 3 or 4+ in 10, 2+ in 4, 1+ in 6) (**Fig 1*g***). The nonsecretory clear cells and myoepithelial cells were negative (**Fig 1*g***).

Compared with the typically strong 15-LOX-2 immunostaining in secretory cells of sebaceous, apocrine, and eccrine glands, there was only weak to moderate immunostaining of keratinocytes in the epidermis in the majority of cases. Staining was generally noted in the basal cells (1+ in 18, 2+ in 15 cases) (**Fig 1*h***), with focal staining in keratinocytes in the stratum spinosum in rare cases. In contrast, the invasive malignant epithelial cells of basal cell carcinoma were uniformly negative (n = 5) (**Fig 2*a***). Considering all cases, no 15-LOX-2 immunostaining was noted in the dermis, dermal inflammatory cells, smooth muscle of erector pili, blood vessels, nevus cells (in a case with an intradermal nevus), nerves, lacrimal glands (n = 1), retinal epithelium (n = 2), ocular ciliary body (n = 2), or conjunctiva (n = 2). As expected from the previous demonstration of 15-LOX-2 mRNA in cornea (Brash *et al*, 1997), strong immunostaining was noted in corneal epithelium (n = 2; not shown).

Possible 15-LOX-2 expression in benign Meibomian glands, sebaceous adenomas, and sebaceous carcinomas In striking contrast to the uniform strong 15-LOX-2 immunostaining in skin sebaceous glands, Meibomian glands of the eyelids were completely negative for 15-LOX-2 (10 sections from nine different patients) (**Fig 2*b*, *c***). In sections with negative Meibomian glands, strong immunostaining for 15-LOX-2 in adjacent cutaneous adnexa (e.g., eccrine or apocrine glands) was present, attesting to preserved antigenicity in individual tissue sections.

15-LOX-2 immunostaining was detected in differentiated cells of four of four cutaneous sebaceous adenomas (**Fig 2*d***); 2+ to 4+ immunostaining was evident in most cells with vacuolated cytoplasm, with negative staining in basal cells or cells with less cytoplasm, generally similar to that of benign sebaceous lobules described above. Immunostaining of a small number of cases of skin sebaceous carcinoma showed variable, reduced 15-LOX-2 expression in neoplastic cells (n = 4) (**Fig 2*e***). One case with strong staining in benign skin sebaceous glands showed markedly reduced to absent staining in neoplastic cells demonstrating *in situ* or Pagetoid spread (not shown). As expected from the lack of 15-LOX-2 immunostaining in benign Meibomian glands, sebaceous



carcinomas of the eyelid and apparently arising from Meibomian glands (n = 12) were generally 15-LOX-2 negative (**Fig 2*f–h***), with eight cases showing complete lack of immunostaining and four cases showing either only faint staining or more definitive immunostaining in < 10% of tumor cells.

15-LOX-2 mRNA in sebaceous glands by *in situ* hybridization *In situ* hybridization was used to identify the cutaneous cell types expressing 15-LOX-2 mRNA and confirm the results of immunohistochemistry. 15-LOX-2 was highly expressed in all sebaceous tissues examined (n = 6) whether from neonatal or adult skin, or from foreskin, breast, or abdominal locations (**Fig 3**). Results obtained from hair follicles sectioned serially indicated that no parts of the hair root sheath, hair, dermal papillae, follicular epidermis, or dermis showed hybridization above background levels. These data confirm the results showing uniform 15-LOX-2 immunoreactivity in cutaneous sebaceous glands and further document the specificity of the antibody employed.

DISCUSSION

In this study, we have demonstrated by immunohistochemistry and *in situ* hybridization the strong and uniform expression of 15-LOX-2 in benign cutaneous sebaceous glands. *In situ* hydridization is highly specific, being capable of distinguishing mRNA sequences that differ by 10%; in other words, all transcripts with less than 90% sequence identity (Keeney, 1999). There are no known human transcripts with over 50% identity to 15-LOX-2 and, therefore, the strong positive *in situ* results are definitive. By immunohistochemistry, the most obvious and strongest expression of 15-LOX-2 was in sebaceous glands, which is completely in accord with the *in situ* results. Furthermore, based on our substantial experience with the immunohistochemistry of 15-LOX-2, including correlation with detection of 15-LOX activity by high-performance liquid chromatography (Shappell *et al*, 1999), the negative immunostaining is highly reliable. The significance of weaker 15-LOX-2 immunostaining in basal layers of the epidermis is discussed below.

15-LOX-2 was expressed in differentiated secretory sebocytes. As in prostate, the differentiation of sebocytes is regulated by androgens (Thody and Shuster, 1989; Luu-The *et al*, 1994; Russell and Wilson, 1994; Deplewski *et al*, 1997). It has proved difficult, however, to induce sebocyte differentiation with androgens *in vitro*. Rosenfield *et al* (1998) postulated a connection between androgens and PPAR receptors. They showed that PPARγ mRNA is present in rat preputial sebocytes and that a synthetic PPARγ agonist induced sebocyte differentiation and that further addition of dihydrotestosterone had an additive effect (Rosenfield *et al*, 1998). In prostate cell lines we have recently demonstrated that 15-HETE activates PPARγ-dependent transcription (Shappell *et al*, 2001). Taken together, these results suggest that 15-HETE may represent an endogenous ligand for PPARγ in 15-LOX-2 expressing tissues, and support a possible correlation of 15-LOX-2 epithelial expression with androgen regulated secretory tissues.

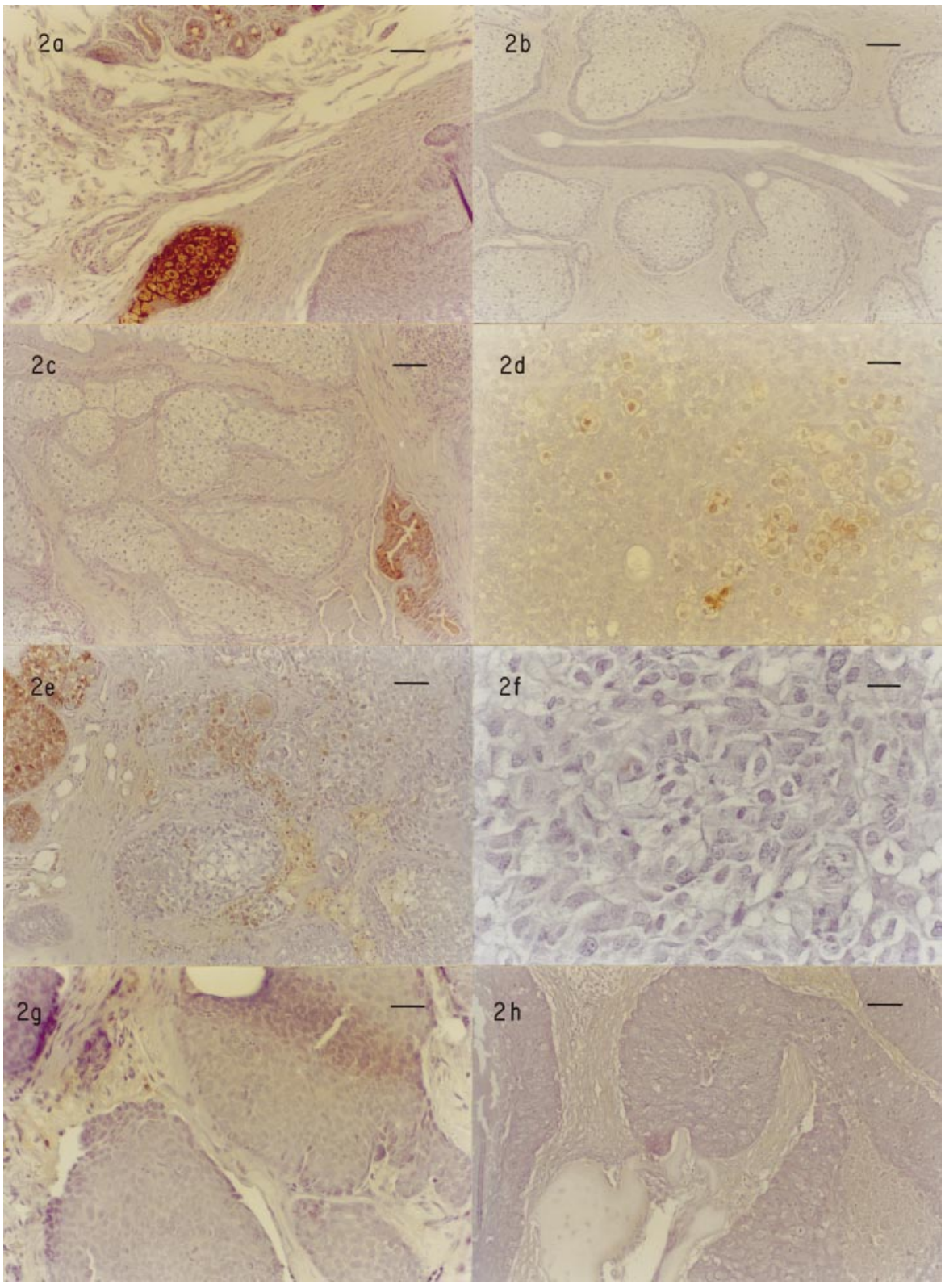
We also detected 15-LOX-2 immunostaining in eccrine and apocrine glands, which are also regulated by androgens (Hay and Hodgins, 1978; Sato *et al*, 1998). Thus, in addition to a possible role in sebocyte differentiation, the localization of 15-LOX-2 in secretory cells of other adnexa (as well as prostatic secretory cells) suggests a general role in regulating secretory differentiation or some aspect of the secretory process. As these different cell types secrete their products by fundamentally different processes, based on the present evidence we would favor a contribution to cellular secretory differentiation rather than a participation in the secretion mechanism *per se*.

The expression of 15-LOX-2 is known to be reduced in prostate adenocarcinomas (Shappell *et al*, 1999; Jack *et al*, 2000). Perhaps similarly, the mouse 8-LOX (the 15-LOX-2 homolog), although upregulated in benign skin squamous papillomas induced by phorbol esters, is absent in squamous cell carcinomas (Lehmann *et al*, 1992; Burger *et al*, 1999). Therefore, it was of interest to determine the possibility of changed 15-LOX-2 expression with the reduced cellular differentiation in sebaceous neoplasms. 15-LOX-2 immunostaining was present in cells with vacuolated cytoplasm, correlating with differentiation, in sebaceous adenomas. Although there was a suggestion of some reduced expression in a small number of cutaneous sebaceous carcinomas, investigation in a larger number of the more common Meibomian gland derived eyelid sebaceous carcinomas revealed the surprising result that benign Meibomian glands are uniformly negative for 15-LOX-2. Meibomian glands are modified sebaceous glands, which also appear to express genes related to androgen processing and signaling, supporting androgen modulation (Perra *et al*, 1990; Sullivan *et al*, 1998). The reason for this striking difference in 15-LOX-2 expression between these related tissues remains to be established. It is possible that tissue specific expression of other genes may be responsible for modulating 15-LOX-2 expression in androgen sensitive tissues. To the best of our knowledge this difference in 15-LOX-2 expression in cutaneous sebaceous glands and Meibomian glands represents the first major biochemical contrast to be reported. This differential expression could partly contribute to pathologic conditions with differential predilection for these two sites, such as acne at cutaneous sites with abundant 15-LOX-2-positive sebaceous glands and the greater frequency of neoplastic transformation of 15-LOX-2-negative Meibomian glands.

In comparison with the strong immunostaining for 15-LOX-2 in sebaceous glands, weaker immunostaining was generally noted in the epidermis. Immunostaining was seen mainly in the less differentiated basal layers. This appears to be in contrast to expression in differentiated secretory cells, such as prostate apical secretory epithelial cells (Shappell *et al*, 1999) and sebocytes. Such basal cell expression was not appreciated in the smaller number of skin samples analyzed by *in situ* hybridization. The only known lipoxygenases that might cross-react with our antibody (see *Materials and Methods*) are the 12R-LOX (Boeglin *et al*, 1998; Sun *et al*, 1998) and a third type of epidermal LOX with unknown positional specificity (Kinzig *et al*, 1999); however, by *in situ* analysis, the 12R-LOX is expressed in differentiated keratinocytes and it has low expression in normal skin with no expression in

Figure 1. 15-LOX-2 immunostaining in benign cutaneous adnexa and epidermis. (*a*) Strong uniform 15-LOX-2 immunostaining in sebaceous glands (top and center) and eccrine glands (left center) from skin of cheek in 17 y old female. Note negative outer root sheath cells (with clear cytoplasm) surrounding some weak staining in follicle (bottom left). (*b*) Typical strong immunostaining in vacuolated cytoplasm of differentiated sebocytes, with negative surrounding undifferentiated cells. (*c*) Strong uniform sebaceous gland 15-LOX-2 immunostaining, with negative follicles, in nevus sebaceous of Jadassohn from scalp of 14 y old male. Note negative outer root sheath adjacent to strongly positive sebaceous lobule, top left. (*d*) High magnification showing uniform cytoplasmic immunostaining of differentiated sebocytes, with abundant cytoplasmic vacuolization paralleling lipid accumulation. Possible nuclear staining is noted focally. (*e*) Uniform 15-LOX-2 immunostaining in apocrine glands (right and bottom) from eyelid of 76 y old female. Note absent staining in outer sheath cells surrounding hair shaft (bottom left) and staining cells within adjacent follicle (top left). (*f*) Higher magnification showing 15-LOX-2 immunostaining of apocrine glands, including apical ''snouts'' and intraluminal secretion (center), from eyelid of 70 y old female. (*g*) 15-LOX-2 immunostaining in luminal secretory cells of eccrine glands, with negative clear cells and outer myoepithelial cells. Note strong immunostaining of adjacent sebaceous gland (right). (*h*) 15-LOX-2 immunostaining in basal cells in epidermis, with some (generally weaker) staining noted focally in suprabasal cells. Note negative papillary dermis. This degree of epidermal staining was stronger than that generally noted. *Scale bars*: (*a*, *c*) 250 μm; (*b*, *e*) 100 μm; (*g*) 50 μm; (*d*, *f*, *h*) 25 μm.

sebaceous glands (unpublished results), so this is unlikely to account for the antigen detected in epidermis by 15-LOX-2 immunostaining or to contribute at all to immunostaining detected in sebaceous glands. Cross-reaction with the third type of epidermal LOX is undetermined. Therefore, we consider the current immunohistochemical results showing epidermal expression to be potentially representative of true 15-LOX-2 expression in epidermis. In the mouse, expression of the murine homolog 8-LOX increases in hyperplastic epidermis, being localized particularly in the granular layer following topical phorbol ester treatment (Fürstenberger *et al*, 1991; Jisaka *et al*, 1997). The 8-LOX may contribute to keratinocyte differentiation in the mouse, potentially activating PPARα by its metabolite 8S-HETE (Muga *et al*, 2000). As described above, expression of the 8-LOX is reduced with



progression to carcinoma (Lehmann *et al*, 1992). In this regard it is intriguing that in contrast to the weak to moderate 15-LOX-2 immunostaining in basal layers of benign epidermis, neoplastic cells of basal cell carcinoma were negative. In general terms, however, the expression patterns of 15-LOX-2 in human skin and the 8-LOX in mouse skin are different (**Table I**). The mouse 8-LOX is present in differentiated keratinocytes with phorbol ester treatment and in follicular epithelium; however, it is not seen in sebaceous glands (unpublished observations), whereas 15-LOX-2 was not generally expressed in differentiated keratinocytes of the epidermis. It should be noted, however, that the mouse 8-LOX is only weakly expressed in normal murine epidermis unless induced by phorbol ester, a condition that that has not been mimicked in the human. Future studies will be needed to determine possible increased 15-LOX-2 expression in benign or reactive epidermal proliferations and the effects of 15S-HETE on keratinocytes.

Finally, given the strong expression of 15-LOX-2 in sebocytes, the possibility that its product 15-HETE contributes to other disease processes of sebaceous glands, such as acne, should be considered. Results of studies done more than three decades ago examining the effects of eicosa-5:8:11:14-tetraynoic acid on sebum production and acne, reinterpreted in light of subsequent knowledge of this compound's inhibitory effects on cyclooxygenases and lipoxygenases, lend *in vivo* support to this hypothesis. Eicosa-5:8:11:14-tetraynoic acid, thought to exert its effects on the inhibition of cholesterol synthesis, resulted in suppression of sebum production in all subjects tested and an improvement in acne in a small number of cases examined (Strauss *et al*, 1967). In subsequent small trials, topical 2% tetraynoic acid in ethanol appeared to reduce sebum secretion in acne patients (Burton and Shuster, 1970; Haroon *et al*, 1970), whereas similar beneficial effects were not seen when topical formulation included 20% propylene glycol in addition to ethanol (Burton and Shuster, 1972; Haroon *et al*, 1972). Eicosa-5:8:11:14-tetraynoic acid is well known to be a nonspecific competitive inhibitor of cyclooxygenases and lipoxygenases. It inhibits 15-LOX-2, albeit not potently (Kilty *et al*,

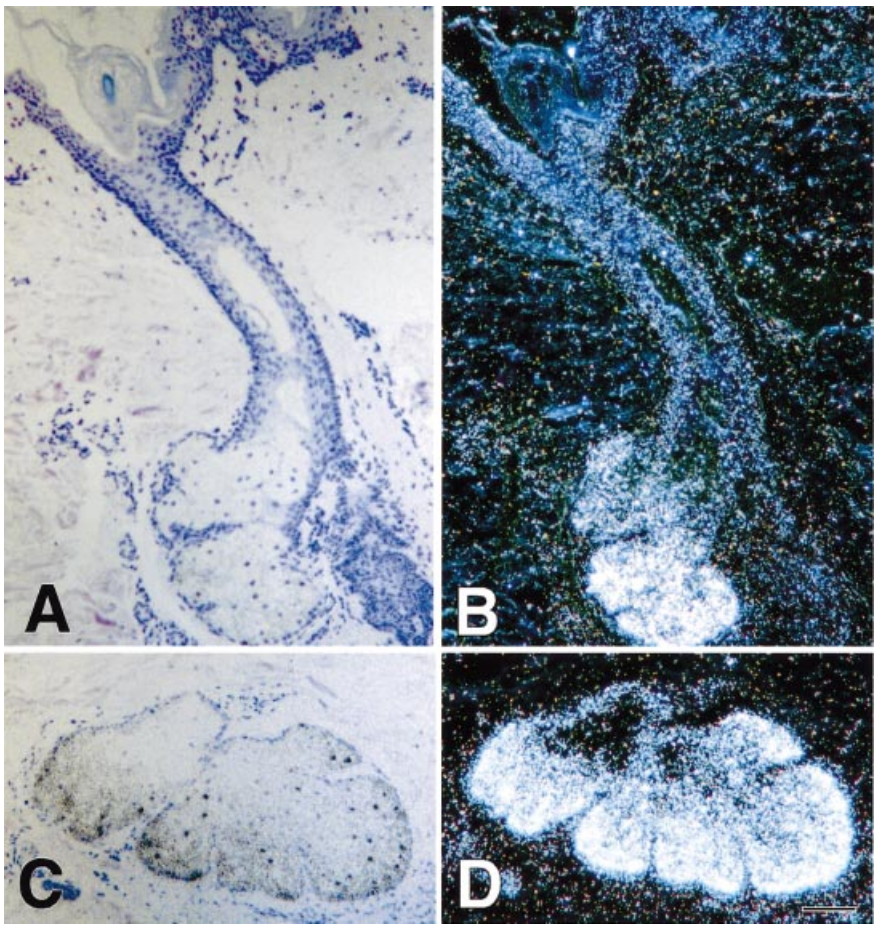


Figure 3. *In situ* localization of 15S-lipoxygenase-2 mRNA in human sebocytes. Neonatal foreskin tissues were sectioned serially and hybridized with sense and anti-sense radiolabeled cRNA specific for 15-LOX-2. Data for sense cRNA are not shown. (*A*, *C*) Tissue morphology is shown in bright field. (*B*, *D*) Specific hybridization of anti-sense cRNA is represented by the silver grains visualized in dark-field illumination. Developed silver grains in the emulsion appear white in dark field and black in bright field. Silver grains were highly concentrated in sebaceous glands but not detected above background levels in other cutaneous cell types. *Scale bar*: 400 μM.

Figure 2. 15-LOX-2 immunostaining in basal cell carcinoma, Meibomian glands, and sebaceous neoplasms. (*a*) Absent 15-LOX-2 in invasive nest of basal cell carcinoma (bottom right). Note positive staining in benign sebaceous gland (bottom center) and eccrine glands (top). (*b*, *c*) Completely negative immunostaining in Meibomian glands (from two different cases). Note staining of adjacent apocrine glands in (*c*). (*d*) Focal moderate 15-LOX-2 immunostaining in cells with more vacuolated cytoplasm in a sebaceous adenoma from the face of a 67 y old female. Note generally absent staining in cells with less cytoplasm. (*e*) Extensively absent 15-LOX-2 immunostaining in sebaceous carcinoma from eyelid of a 40 y old white female. Adjacent tissues suggested origin from cutaneous sebaceous glands. Note absent staining in many cells with abundant clear cytoplasm (bottom and right). Focal strong staining is noted in other portions of the neoplasm (top left). (*f*) Negative 15-LOX-2 immunostaining in atypical cells of a sebaceous carcinoma from the left orbit (Meibomian gland derived) of a 92 y old female. (*g*) Absent 15-LOX-2 immunostaining in poorly differentiated sebaceous carcinoma from eyelid of 75 y old female. (*h*) Negative 15-LOX-2 immunostaining in a sebaceous carcinoma (Meibomian gland derived) invading the orbit of a 70 y old female. Note trabecular bone bottom left. *Scale bars*: (*a–c*, *e*, *h*) 100 μm; (*d*, *g*) 50 μm; (*f*) 25 μm.

Table I. Comparison of structure, function, and location of human 15-LOX-2 and murine 8-LOX

	Human 15-LOX-2	Mouse 8-LOX	References
% Amino acid identity	78%	78%	Jisaka *et al* (1997)
Substrate specificity	AA > LA	AA > LA	Brash *et al* (1997, 1999a), Kilty *et al* (1999), Burger *et al* (1999)
Product from AA	15*S*-HPETE	8*S*-HPETE	Brash *et al* (1997), Jisaka *et al* (1997), Kilty *et al* (1999), Burger *et al* (1999)
Product from LA	13*S*-HPODE	9*S*-HPODE	Brash *et al* (1997), Jisaka *et al* (1997), Kilty *et al* (1999), Burger *et al* (1999)
Tissue localization			
Prostate	Present	Absent[a]	Brash *et al* (1997), Shappell *et al* (1999)
Lung	Present	Not reported	Brash *et al* (1997), Kilty *et al* (1999)
Cornea	Present	Not reported	Brash *et al* (1997)
Skin	Present	Present	Brash *et al* (1997), Jisaka *et al* (1997), Burger *et al* (1999), Heidt *et al* (1900)
Brain	Present[b]	Present[c]	Brash *et al* (1997), Jisaka *et al* (1997)
Distribution in skin			
Sebaceous glands, other adnexa	Present	Absent[d]	Current study
Epidermis	Present (basal layer)	Present[e] (differentiated keratinocytes; hair follicle)	Current study; Gschwendt *et al* (1986), Jisaka *et al* (1997), Heidt *et al* (2000)

AA, arachidonic acid.
[a]Shappell, S.B., Brash, A.R., unpublished observations.
[b]Absent by Northern blot on total tissue RNA (multitissue northerns) (Brash 1997); focal by immunohistochemistry (Shappell, S.B., Brash, A.R., unpublished observations).
[c]Distribution in brain not established.
[d]Shappell, S.B., Keeney, D.S., Brash, A.R., unpublished observations.
[e]Upregulated by phorbol esters, which has not been tested in humans.

1999). It is thus interesting to speculate that the real mechanism of reduced sebaceous gland activity by eicosa-5:8:11:14-tetraynoic acid might be via 15-LOX-2 inhibition and that 15-LOX-2 inhibitors might represent potential novel anti-acne pharmacologic agents. The recognition of 15-LOX-2- and PPARγ-dependent pathways in androgen-regulated tissues raises important considerations of possible interactions of these signaling mechanisms in regulating differentiation in 15-LOX-2 expressing tissues.

Supported in part by Department of Defense Grant DAMD 17–98–1-8637 (S.B.S.), a Discovery Grant from the Vanderbilt-Ingram Cancer Center (S.B.S., A.R.B.), a VUMC Intramural Interdisciplinary Grant (S.B.S.), NIH/NIAMS AR02002 (D.S.K.) and AR41943 (D.S.K.), the Dermatology Foundation (D.S.K.), and NIH GM-53638 (A.R.B.). The authors wish to thank Caroline Nolen and the Vanderbilt University Medical Center Histology Laboratory for cutting sections for immunohistochemical staining and Dr David Page for useful comments and discussion.

REFERENCES

Baer AN, Green FA: Fatty acid oxygenase activity of human hair roots. *J Lipid Res* 34:1505–1514, 1993

Baer AN, Costello PB, Green FA: In vivo activation of an ω-6 oxygenase in human skin. *Biochem Biophys Res Commun* 180:98–104, 1991

Boeglin WE, Kim RB, Brash AR: A 12R-lipoxygenase in human skin: Mechanistic evidence, molecular cloning, and expression. *Proc Natl Acad Sci USA* 95:6744–6749, 1998

Brash AR: Lipoxygenases: Occurrence, functions, catalysis, and acquisition of substrate. *J Biol Chem* 274:23679–23682, 1999

Brash AR, Boeglin WE, Chang MS: Discovery of a second 15S-lipoxygenase in humans. *Proc Natl Acad Sci USA* 94:6148–6152, 1997

Brash AR, Jisaka M, Boeglin WE, Chang MS: Molecular cloning of a second human 15S-lipoxygenase and its murine homologue, an 8S-lipoxygenase. Their relationship to other mammalian lipoxygenases. *Adv Exp Med Biol* 447:29–36, 1999a

Brash AR, Jisaka M, Boeglin WE, et al: Investigation of a second 15S-lipoxygenase in humans and its expression in epithelial tissues. *Adv Exp Med Biol* 469:83–89, 1999b

Burrall BA, Cheung M, Chiu A, Goetzl EJ: Enzymatic properties of the 15-lipoxygenase of human cultured keratinocytes. *J Invest Dermatol* 91:294–297, 1988

Burger F, Krieg P, Kinzig A, Schurich B, Marks F, Fürstenberger G: Constitutive expression of 8-lipoxygenase in papillomas and clastogenic effects of lipoxygenase-derived arachidonic acid metabolites in keratinocytes. *Mol Carcinog* 24:108–117, 1999

Burrall BA, Wintroub BU, Goetzl EJ: Selective expression of 15-lipoxygenase activity by cultured human keratinocytes. *Biochem Biophys Res Commun* 133:208–213, 1985

Burton JL, Shuster S: Topical tetraynoic acid and sebum excretion. *Br J Dermatol* 82:626–627, 1970

Burton JL, Shuster S: The effect of topical tetraynoic acid on the seborrhoea of acne. *Br J Dermatol* 86:66–67, 1972

Deplewski D, Liao S, Rosenfield RL: Preputial sebocyte 5alpha-reductase isoform specificity. *Endocrinology* 138:4416–4420, 1997

Duell EA, Ellis CN, Voorhees JJ: Determination of 5,12, and 15-lipoxygenase products in keratomed biopsies of normal and psoriatic skin. *J Invest Dermatol* 91:446–450, 1988

Fürstenberger G, Hagedorn H, Jacobi T, Besemfelder E, Stephan M, Lehmann WD, Marks F: Characterization of an 8-lipoxygenase activity induced by the phorbol ester tumor promoter 12-O-tetradecanoylphorbol-13-acetate in mouse skin in vivo. *J Biol Chem* 266:15738–15745, 1991

Green FA: Generation and metabolism of lipoxygenase products in normal and membrane-damaged cultured human keratinocytes. *J Invest Dermatol* 93:486–491, 1989

Gschwendt M, Fürstenberger G, Kittstein W, et al: Generation of the arachidonic acid metabolite 8-HETE by extracts of mouse skin treated with phorbol ester in vivo; identification by 1H-n.m.r. & GC-MS spectroscopy. *Carcinogenesis* 7:449–455, 1986

Haroon TS, Hall J, Lyell A, Alexander JOD: Topical tetraynoic acid. *Br J Dermatol* 83:418–419, 1970

Haroon TS, Hall J, Haroon A: Effect of topical tetraynoic acid on sebum excretion. *Br J Dermatol* 86:64–65, 1972

Hay JB, Hodgins MB: Distribution of androgen metabolizing enzymes in isolated tissues of human forehead and axillary skin. *J Endocrinol* 79:29–39, 1978

Heidt M, Furstenberger G, Vogel S, Marks F, Krieg P: Diversity of mouse lipoxygenases: identification of a subfamily of epidermal isozymes exhibiting a differentiation-dependent mRNA expression pattern. *Lipids* 35:701–707, 2000

von Henneicke-Zepelin HH, Schröder J-M, Smíd P, Reusch MK, Christophers E:

Metabolism of arachidonic acid by human epidermal cells depends on the maturational stage. *J Invest Dermatol* 97:291–297, 1991

Hughes MA, Brash AR: Investigation of the mechanism of biosynthesis of 8-hydroxyeicosatetraenoic acid in mouse skin. *Biochim Biophys Acta* 1081:347–354, 1991

Jack GS, Brash AR, Olson SJ, Manning S, Coffey CS, Smith JA Jr, Shappell SB: Reduced 15-lipoxygenase-2 immunostaining in prostate adenocarcinoma: Correlation with grade and expression in high grade prostatic intraepithelial neoplasia. *Hum Pathol* 31:1146–1154, 2000

Jisaka M, Kim RB, Boeglin WE, Nanney LB, Brash AR: Molecular cloning and functional expression of a phorbol ester-inducible 8S-lipoxygenase from mouse skin. *J Biol Chem* 272:24410–24416, 1997

Keeney DS. Radiolabeled cRNA and in situ hybridization. In: Sundberg JP, Bogess D (eds). *Systematic Approach to Evaluation of Mouse Mutations*. Boca Raton: CRC Press, 1999, pp 145–167

Kilty I, Logan A, Vickers PJ: Differential characteristics of human 15-lipoxygenase isozymes and a novel splice variant of 15S-lipoxygenase. *Eur J Biochem* 266:83–93, 1999

Kinzig A, Heidt M, Fürstenberger G, Marks F, Krieg P: cDNA cloning, genomic structure, and chromosomal localization of a novel murine epidermis-type lipoxygenase. *Genomics* 58:158–164, 1999

Krieg P, Kinzig A, Heidt M, Marks F, Fürstenberger G: cDNA cloning of a 8-lipoxygenase and a novel epidermis-type lipoxygenase from phorbol ester-treated mouse skin. *Biochim Biophys Acta* 1391:7–12, 1998

Lehmann WD, Stephan M, Fürstenberger G: Profiling assay for lipoxygenase products of linoleic and arachidonic acid by gas chromatography-mass spectrometry. *Anal Biochem* 204:158–170, 1992

van Leyen K, Duvoisin RM, Engelhardt H, Wiedmann M: A function for lipoxygenase in programmed organelle degradation. *Nature* 395:392–395, 1998

Luu-The V, Sugimoto Y, Labrie Y, Solache I, Singh M, Labrie F: Characterization, expression, and immunohistochemical localization of 5alpha-reductase in human skin. *J Invest Dermatol* 102:221–226, 1994

Muga SJ, Thuillier P, Pavone A, et al: 8S-lipoxygenase products activate peroxisome proliferator-activated receptor alpha and induce differentiation in murine keratinocytes. *Cell Growth Differ* 11:447–454, 2000

Nagy L, Tontonoz P, Alvarez JGA, Chen H, Evans RM: Oxidized LDL regulates macrophage gene expression through ligand activation of PPARgamma. *Cell* 93:229–240, 1998

Nugteren DH, Kivits GAA: Conversion of linoleic acid and arachidonic acid by skin epidermal lipoxygenases. *Biochim Biophys Acta* 921:135–141, 1987

Pelletier G, Luu-The V, Huang X, Lapointe H, Labrie F: Localization by in situ hybridization of steroid 5alpha-reductase isozyme gene expression in the human prostate and preputial skin. *J Urol* 160:577–582, 1998

Perra MT, Lantini MS, Serra A, Cossu M, De Martini G, Sirigu P: Human Meibomian glands: a histochemical study for androgen metabolic enzymes. *Invest Ophthalmol Vis Sci* 31:771–775, 1990

Rosenfield RL, Deplewski D, Kentsis A, Ciletti N: Mechanisms of androgen induction of sebocyte differentiation. *Dermatology* 196:43–46, 1998

Russell DW, Wilson JD: Steroid 5alpha-reductase: two genes/two enzymes. *Annu Rev Biochem* 63:25–61, 1994

Sato T, Sonoda T, Itami S, Takayasu S: Predominance of type I 5alpha-reductase in apocrine sweat glands of patients with excessive or abnormal odour derived from apocrine sweat (osmidrosis). *Br J Dermatol* 139:806–810, 1998

Schewe T, Kühn H: Do 15-lipoxygenases have a common biological role? *Trends Biochem Sci* 16:369–373, 1991

Shappell SB, Boeglin WE, Olson SJ, Kasper S, Brash AR: 15-lipoxygenase-2 (15-LOX-2) is expressed in benign prostatic epithelium and reduced in prostate adenocarcinoma. *Am J Pathol* 155:235–245, 1999

Shappell SB, Gupta RA, Manning S, *et al:* 15S-hydroxyeicosatetraenoic acid activates peroxisome proliferator-activated receptor γ and inhibits proliferation in PC3 prostrate carcinoma cells. *Cancer Res* 61:497–503, 2001

Strauss JS, Pochi PE, Whitman EN: Suppression of sebaceous gland activity with eicosa-5:8:11:14-tetraynoic acid. *J Invest Dermatol* 48:492–493, 1967

Sullivan DA, Rocha EM, Ullman MD, et al: Androgen regulation of the Meibomian gland. *Adv Exp Med Biol* 438:327–331, 1998

Sun D, McDonnell M, Chen X-S, et al: Human 12 (R) -lipoxygenase and the mouse ortholog. Molecular cloning, expression, and gene chromosomal assignment. *J Biol Chem* 273:33540–33547, 1998

Thody AJ, Shuster S: Control and function of sebaceous glands. *Physiol Rev* 69:383–416, 1989

Zhao H, Richards-Smith B, Baer AN, Green FA: Lipoxygenase mRNA in cultured human epidermal and oral keratinocytes. *J Lipid Res* 36:2444–2449, 1995